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Factors affecting inundative releases of *Trichogramma minutum* Ril. against the Spruce Budworm[1]

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Abstract

Inundative releases of *Trichogramma minutum* Riley for biological control of the spruce budworm, *Choristoneura fumiferana* (Clemens), were investigated in Canada's boreal forest during 1982 and 1983. The most significant factors affecting the level of egg mass parasitism were the time of release, the density of the parasitoids, and the local weather conditions. Food supply to the female parasitoids, vertical location of the host egg mass in the stand, intensity of solar radiation, and density of the host insect were less important. Parasitism was not affected by the tree species on which budworm egg masses were laid. Parasitoids reared at different temperatures and on different host eggs differed in biological characteristics and could influence the success of field releases. Geographical strains of *T. minutum* were not considered as important in subsequent releases as the rearing conditions because of the high degree of individual variation within each strain.

1 Introduction

In Canada's Eastern boreal forest, the spruce budworm, *Choristoneura fumiferana* (Clemens), is considered the most damaging insect pest. As of September 1983, 12.1 million hectares of forest within one province alone, Ontario, had suffered tree mortality as a direct result of budworm infestations (Anonymous 1983). Currently, the spruce budworm is controlled by selective

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aerial applications of insecticides. The continued, exclusive reliance on insecticides as the principal, long-term, management technique, however, is unacceptable environmentally and socially. With advancements in technology over the past decade, biological control of the spruce budworm through mass production in the laboratory and field inundation of parasitoids has potential as an alternative to chemical insecticides (KNIPLING 1977).

Egg parasitoids are considered attractive species for biological control of forest pests because: 1. they have few hyperparasites or diseases; 2. they can have a significant impact upon a pest population before the damaging life stage; and 3. they are easy to rear and mass produce (ANDERSON 1976). *Trichogramma minutum* Riley is the only known parasitoid that attacks eggs of the spruce budworm in Eastern North America (MILLER 1963). Budworm eggs are parasitized and destroyed within the first four days of oviposition, making this parasitoid an attractive species for biological control (HOUSEWEART et al. 1982).Generally, *T. minutum* is not important in the natural control of the spruce budworm since it is polyphagous and dependent upon alternate hosts for most of its life cycle (JENNINGS and HOUSEWEART 1980). Manipulation of this species, however, may be possible in the forest environment; natural levels of parasitism, although normally less than 15 %, have been reported as high as 77 %, and both tree density and stand composition have been shown to affect percentage parasitism (KEMP and SIMMONS 1978; ANDERSON 1976).

Historically, the effectiveness of *Trichogramma* species in controlling pest populations has produced conflicting results (BILIOTTI 1977). One of the reasons for this has been the difficulty in understanding the influence of various conditions at the time of the release. In view of this, and the need to find an alternative to chemical controls in the forest ecosystem, a project was initiated in 1982 to investigate the feasibility of using inundative releases of *T. minutum* against the spruce budworm (CARROW et al. 1984). In particular, we have tried to identify those factors which could affect the outcome of such releases. We present, here, an overview of the project, summarizing those factors, both environmental and rearing, which we believe to be of prime consideration in future inundative releases. A more detailed description and discussion on the feasibility of this approach for control of the spruce budworm will be given in a subsequent publication.

2 Materials and methods

The field research was conducted in Central Canada near Hearst, Ontario (50° N, 84° W). Local weather conditions, including maximum and minimum temperatures, relative humidity, rainfall, number of sunshine hours and wind speed and direction, were monitored, daily, throughout the study. Sample plots, 0.25 ha in size, were established in young, white spruce (*Picea glauca* [Moench] Voss) plantations (18 yr), containing 50 % balsam fir (*Abies balsamea* [L.] Mill.) at the time of the study. This area was selected because of the high natural population of spruce budworm (200 egg masses/9.29 m branch) and low level of natural parasitism by *T. minutum* (4 % egg masses parasitized).

Ground releases of *T. minutum* were carried out in the plots on 8 and 14 July 1982 and 7 and 21 July 1983. In 1982, the parasitoids were reared on eggs of *Ephestia kuehniella* by the Biological Control Center at Guelph, Ontario, Canada, while in 1983, they were reared on eggs of *Sitotroga cerealella* by Rincon Vitova, Oak View, California, U.S.A. Host eggs, containing pupae of *T. minutum*, were programmed for release on the specific dates before being glued to cardboard strips and shipped to the field. At the release sites, the cards were cut into the appropriate sizes and placed inside triangular, paper cups with openings at both the top and bottom. The cups were then

pinned at 0.25 m above ground level to vertical stakes located in a 7 × 7 m grid pattern throughout each release plot.

Parasitism was monitored using a continuous supply of fresh, laboratory-reared egg masses from spruce budworm (sentinel egg masses). These were tied to randomly selected trees in each plot, at three heights on each tree; 1.25, 2.25, and 3.25 m above ground. They were replaced every 3 to 4 days throughout the season from mid-June until mid-August. Egg masses collected from the field were placed in individual gelatin capsules (size 00) and held at field temperature until the parasitized eggs had turned black. After 10 days, under these conditions, the number of egg masses parasitized was counted.

To investigate the effect of various factors in the field, cage studies were conducted in the same area. A buffer zone of at least one kilometer was maintained between these studies and the release plots previously described. Cages were constructed of fine-mesh organdy overlaid and glued to a cylinder of chicken wire. Each cage was tied by one end on to a branch of either balsam fir or white spruce and supported by a wooden stake directly beneath. Various densities of sentinel egg masses and newly-emerged *T. minutum* females (less than 6 h old) reared under different conditions or from different geographical areas were then introduced before sealing. The cages were maintained for 4 days, after which the egg masses were returned to the laboratory. A minimum of 10 replicates were used. As before, the egg masses were held separately in individual capsules until parasitism rates could be determined. Results were compared using either a paired Students' 't' Test or Duncan's New Multiple Range Test.

In the laboratory, the effect of rearing temperature and host egg on *T. minutum* were also examined. Unless otherwise specified, parasitoids were maintained at 25 °C, 75 % relative humidity, and 16 : 8 light : dark. To investigate the factor of rearing temperature, parasitoids were reared on eggs of *E. kuehniella* at four different temperatures; 25, 20, 17, and 15 °C under a light regime of 16 : 8 light : dark. The relative humidity was maintained at 75 % using a saturated NaCl solution (WINSTON and BATES 1960). Within 4 hours of emergence, 10 females, from each temperature, were isolated into individual, 1/2-dram vials, provided with a honey solution and a daily supply of fresh *E. kuehniella* eggs until their death.

The effect of different host eggs on *T. minutum* was examined by rearing parasitoids either on eggs of *E. kuehniella* or *C. fumiferana*. Within 4 hours of emergence, 30 female parasitoids from each host were isolated in individual, 1/2-dram vials and provided, daily, with a fresh supply of *E. kuehniella* eggs. The sex ratio, emergence, longevity, and daily and total fecundity per female was determined for parasitoids reared at each temperature and from each host egg. Students' 't' Test or Duncan's New Multiple Range Test were used for the analyses.

3 Results and discussion

Results from the field releases in 1982 and 1983 indicate that one of the key factors in the subsequent level of parasitism of spruce budworm egg masses is the number of parasitoids released. In 1982, a rate of 480,000 *T. minutum* females/ha was applied to one release plot. Maximum parasitism of sentinel egg masses following this release was 16 %. In 1983, 12 million female *T. minutum* females/ha were released with maximum parasitism of 87 % of the egg masses. Increasing the application rate twenty-five times, therefore, resulted in only a five-fold increase in parasitism. This suggests that the relationship between the rate of release and parasitism of spruce budworm egg masses is not linear. Cage studies in the field verified these findings (fig. 1). When 3 egg masses/cage were exposed to densities of 3, 9, 15, 22, and 30 female *T. minutum* for three days, a sigmoid relationship between parasitoid density and parasitism was observed. This was true for both the number of egg masses and the number of eggs being parasitized.

In 1983, one study plot received a single, early release on 8 July while another received one late release on 21 July, each at a rate of 12 million female parasitoids per hectare (fig. 2). Initial parasitism of the sentinel egg masses following the releases, in both cases, was greater than 80 %, as represented by the first peak in each curve. Approximately 14 days later, a second peak in each

plot was observed, representing the second generation of *T. minutum* emerging from natural spruce budworm eggs in the field. The height of this second peak provides some indication as to the density of naturally-laid, acceptable spruce budworm egg masses present in the field at the time of the release. The fact that the second peak in parasitism following the release on 8 July was 90 %, indicates that there were a large number of natural egg masses present. Where parasitoids were released on 21 July, however, the second peak of

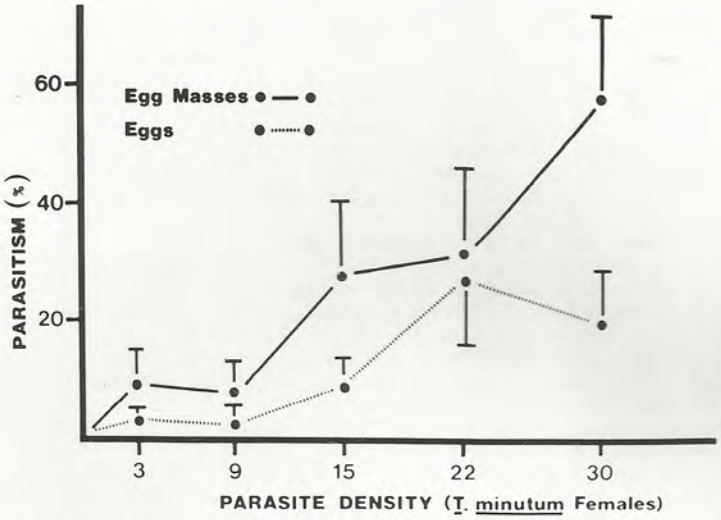

Fig. 1. Mean parasitism of spruce budworm egg masses on branches of balsam fir in field cages by various densities of *Trichogramma minutum*. Standard errors are indicated as bars above each mean, but also represent equal variation below the mean

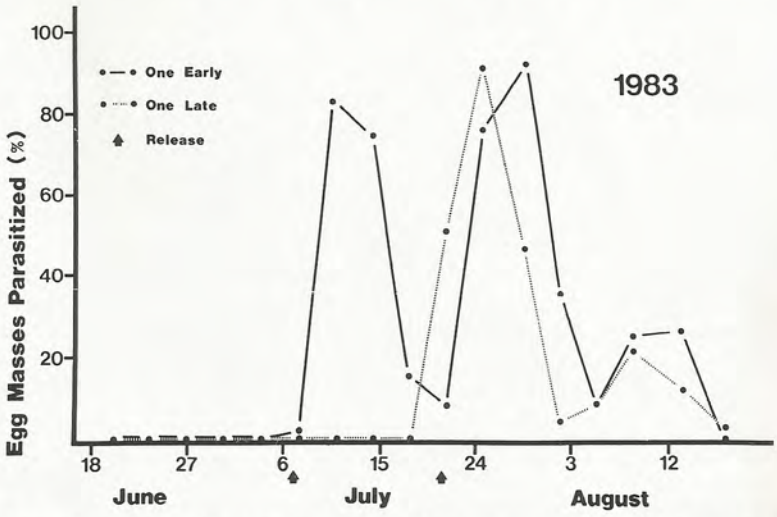

Fig. 2. Seasonal parasitism of sentinel egg masses by *Trichogramma minutum* following inundative release on 8 and 21 July 1983 in a white spruce plantation in Ontario, Canada. Each point represents the percentage of parasitized egg masses from a sample size of 51

parasitism was less than 30 %. Essentially, therefore, the release on 21 July missed the oviposition curve of the budworm whereas that on 8 July was well-synchronized with budworm egg-laying and had a more significant impact on the future number of viable eggs.

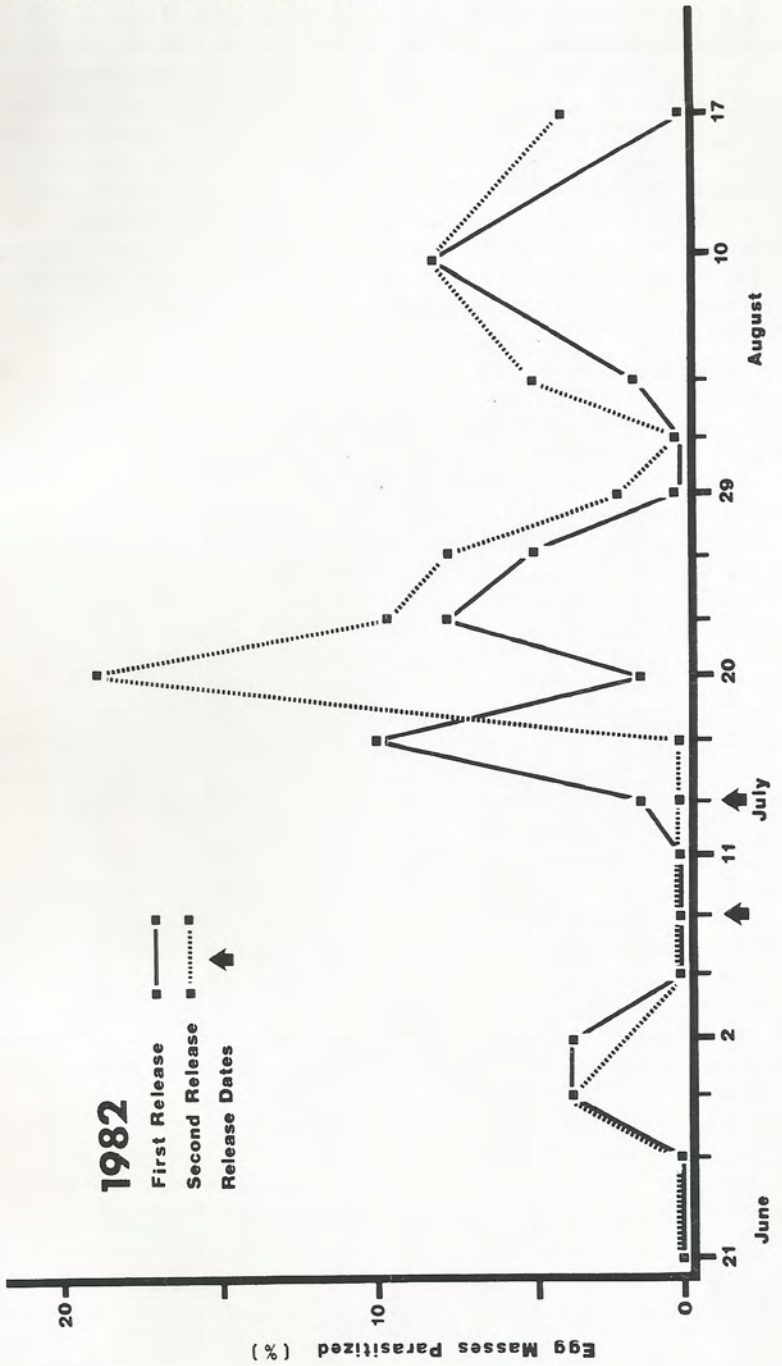

Fig. 3. Seasonal parasitism of sentinel egg masses by *Trichogramma minutum* following inundative release on 7 and 14 July 1982 in a white spruce plantation in Ontario, Canada. Each point represents the percentage of parasitized egg masses from a sample size of 48

In 1982, one plot each received either one early release on 7 July or one late release on 14 July (fig. 3). The effect of timing for the release, could not be determined here since unfavourable weather conditions, after 7 July, delayed and extended emergence of the parasitoids into that of the later release. For one week during and immediately following this release, 81 mm of rainfall and an average minimum temperature of 8 °C were recorded. Under these conditions, emergence of the parasitoids in the field averaged 34 %. During the second release, the total rainfall reported was 17 mm with an average minimum temperature of 11 °C. Emergence of the parasitoids, in this case, averaged 80 %. Although different rates of emergence were observed for each release, the actual number of *T. minutum* released on each plot in 1982 was similar; 240,000 *T. minutum* females/ha. From fig. 3, it is apparent, therefore, that unfavourable weather conditions during the early release, not only affected emergence of the parasitoids but also the level of parasitism. Maximum parasitism of the sentinel egg masses following the early release was approximately 10 % while that following the later release 19 %.

Our sampling design, in the field studies, enabled us to study the effect of two other variables on parasitism by *T. minutum*, namely, the species of host tree and the vertical placement of the egg mass in the canopy. No effect of host tree on parasitism was observed. The total number of parasitized egg masses collected on balsam fir/white spruce in 1982 and 1983 were 51/48 and 514/519, respectively. Similar results were obtained using sleeve cage experiments in the field. The percentage parasitism of eggs and egg masses on balsam fir was 47.0 % (± 8.4 %) and 8.0 % (± 1.8 %), respectively, while that on white spruces was 42.7 % (± 7.8 %) and 6.7 % (± 1.4 %), respectively. Differences in the number of parasitized egg masses were observed, however, with respect to height in the canopy (table 1). In both years, the greatest number of parasitized egg masses were collected from the upper canopy (3.25 m) in all release plots. This pattern is more obvious in 1982 where the rate of release was lower than in 1983 and the area of the release was not saturated with parasitoids. Since the average height of these stands was 5.5 m, our results suggest that *T. minutum* will move into the upper canopy (3.25 m) even when released at ground level (0.25 m).

Results from field cages indicated that food supply to the parasitoid, solar radiation, and the geographical origin of the strain of *Trichogramma* could influence subsequent levels of parasitism. Ten replicates of 15 female *T. minutum* were each provided with a honey solution upon emergence while an equal number were deprived of food. When exposed to 3 egg masses/cage for a period of four days, those parasitoids supplied with food parasitized 38.1 % of the egg masses. Unfed *T. minutum* parasitized only 19.0 % of the egg masses. These results were significantly different at $p < 0.05$. Under similar cage

Table 1. Total number of sentinel egg masses collected by height during inundative releases in 1982 and 1983 in Ontario, Canada

Height of egg mass on tree (m)	No. egg masses parasitized	
	1982	1983
3.25	69	579
2.25	14	520
1.25	17	410

conditions, without a source of food, parasitoids exposed to a level of solar radiation at 6585 lux (± 215 lux) parasitized only 2.1 % (± 1.6 %) of the eggs and 6.6 % (± 4.4 %) of the egg masses while those exposed to a level of 1550 lux (± 307 lux) parasitized 12.4 % (± 5.0 %) of the eggs and 29.9 % (± 7.8 %) of the egg masses in each cage. In addition, differences were observed between *T. minutum* collected from six different geographical regions in eastern North America: Grundy (G), Plummer (P), Rogers (R), and Strong (S) from Northern Ontario, Canada; Vineland (V) from Southern Ontario, Canada, and Maine (M) from the eastern United States. Fifteen, newly-emerged females from each strain were placed in individual cages with 3 egg masses for a period of four days. In all cases, the Plummer strain parasitized significantly more egg masses under field conditions than either Grundy, Strong or Vineland strains (fig. 4). In the laboratory, although differences are also seen, there is considerable within-strain variation and, as yet, the results are not consistent enough to predict the efficacy of strains in the field.

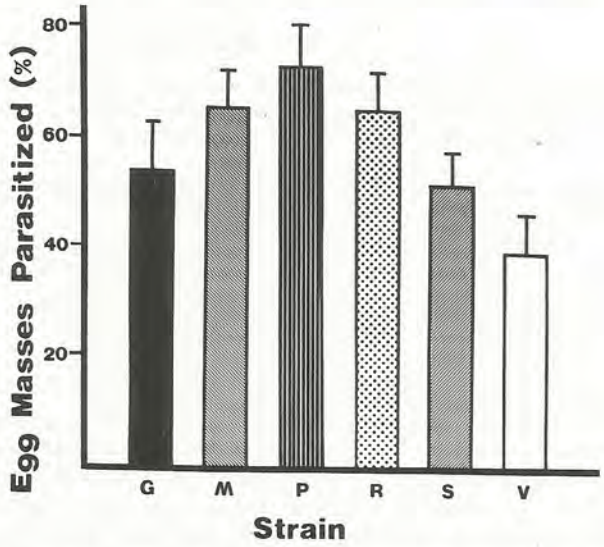

Fig. 4. Parasitism by geographical strains of *Trichogramma minutum* of spruce budworm egg masses on branches of white spruce in field cages. G = Grundy, M = Maine, P = Plummer, R = Rogers, S = Strong, and V = Vineland

Field cages were also used to study the effect of varying host density on parasitism. Within each cage, 15 newly-emerged female *T. minutum* were placed with either 1, 3, 6, 9 or 12 spruce budworm egg masses. Figure 5 shows the functional response of *T. minutum* as the host density changes under field conditions. Clearly, as the number of host egg masses increased up to nine per cage, the number of parasitized egg masses also increased. Using per cent parasitism as the parameter, however, this functional response is much less obvious. Considerable variation was observed at each host density and a significant increase in the per cent parasitism was not seen above a density of three egg masses per cage. The functional relationship between *T. minutum* and the number of spruce budworm egg masses, therefore, appears to be

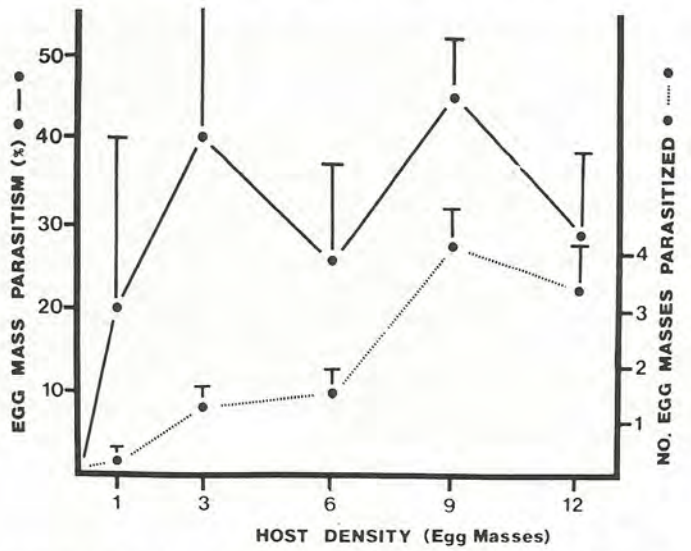

Fig. 5. Mean parasitism by *Trichogramma minutum* at various densities of spruce budworm eggs and egg masses on branches of balsam fir in field cages. Standard errors are indicated as bars above each mean, but also represent equal variation below the mean

independent. Similar results, using other species in field studies, were obtained by WAAGE (1984) and MORRISON and STRONG (1980).

Results from laboratory studies suggest that both the rearing temperature and the host egg will influence the effectiveness of *T. minutum*. Parasitoids reared at lower temperatures produce a greater proportion of female offspring (table 2). Both emergence and the number of eggs laid on the first day of life, however, were reduced. Although not significant, a slight reduction in longevity with a reduction in rearing temperature was also observed. This reduction, as well as the reduction in egg-laying immediately following emergence, is of particular importance in the field where parasitoids are not supplied with a source of food when released and, consequently, can be expected to live a much shorter time. In addition, parasitoids reared from eggs of spruce budworm laid three times as many eggs/female, produced three times as many progeny/female, and lived twice as long as those reared from eggs of *E.*

Table 2. Differences in sex ratio, emergence, longevity, total fecundity, first day fecundity, and total progeny for *T. minutum* reared at 25, 20, 17, and 15 °C

Biological factor	Rearing temperature (°C)			
	25	20	17	15
Sex ratio (% females)	36.4a*	38.4a	54.5b	69.3b
Emergence (%)	84.8a	80.4ab	77.1ab	73.7b
Longevity (days)	21.0a	19.0a	21.1a	16.9a
Total fecundity (eggs/female)	128.0a	92.2a	95.2a	110.6a
First day fecundity (eggs/female)	30.8a	25.4ab	11.9bc	8.8c
Total progeny (number/female)	84.5a	72.5a	78.2a	102.0a

* Means within the same line, followed by different letters are significantly different at $p \leq 0.05$.

kuehniella (table 3). Although such findings, as suggested by BARBER (1937), probably reflect the relatively larger size, and thus, the greater biological capacity of females reared from eggs of spruce budworm, they have important consequences in the efficacy of field releases. Any reduction in key biological characteristics will reduce, undoubtedly, the parasitic potential of these parasitoids released in the field.

Table 3. Differences in sex ratio, emergence, longevity, total fecundity, and total progeny for *T. minutum* reared on spruce budworm, *Choristoneura fumiferana* and Mediterranean flour moth, *Ephestia kuehniella*

Biological factor	*C. fumiferana*	*E. kuehniella*
Sex ratio (% females)	78.0a*	82.2a
Emergence (%)	53.2a	63.6a
Longevity (days)	2.2a	1.0b
Total fecundity (eggs/female)	50.0a	17.3b
Total progeny (number/female)	50.0a	14.1b

* Means within the same line, followed by different letters are significantly different at $p \leq 0.05$.

4 Conclusions

This study shows that the most significant factors affecting inundative release are the number of parasitoids released, the time of the release(s), and the local weather conditions. The relationship between parasitoid density and the subsequent level of parasitism is curvi-linear. Any increase in the desired level of parasitism must be weighed against a more rapid increase in the number of parasitoids required, and thus, the cost of production. It is important that releases of *T. minutum* be closely synchronized with the oviposition period of the spruce budworm. Attempts must be made to ensure that the early stages of oviposition can be accurately predicted and that the scheduling of parasitoid releases remain somewhat sensitive to any changes in oviposition. Clearly, better weather conditions will improve the impact of *T. minutum*, increasing both the rate of parasitism and emergence. This, in turn, will compress the activity of the parasitoids in time. Of no importance, however, will be the species of host tree on which spruce budworm lays its eggs.

The remaining factors will have variable impacts on inundative releases of *T. minutum*. The density-independent relationship of parasitism and the density of the host egg masses makes the density of *T. minutum* more important to the subsequent success of releases than the density of the budworm. A significantly greater proportion of egg masses will be parasitized by *T. minutum* in the upper canopy. This will improve the chances of successful control with inundative releases since a greater number of spruce budworm egg masses are concentrated in this zone. In addition, a lower level of parasitism could be expected in stands with lower stocking densities since greater parasitism was observed at lower light intensities. Supplying food to the parasitoid upon emergence will definitely increase the vigour and subsequent parasitism of *T. minutum*, however, the cost of developing such technology probably cannot justify this approach, at the present.

Strains of *T. minutum* from different geographical regions could affect levels of parasitism in the field. Current difficulty in translating laboratory results to the field make further investigations in this area of prime importance. Finally, rearing conditions, such as low temperature, which are in standard use for synchronizing the emergence of *Trichogramma* species in mass production, will affect parasitism in the field. Because of the short life span of parasitoids in the field, these effects should be weighed carefully against the benefits of highly synchronized emergence. In addition, although parasitoids reared from the target host, spruce budworm, are biologically more efficient than those from the standard rearing host, it is unlikely, technologically, that mass production of spruce budworm can be used for releases of *T. minutum*. These findings can be important, however, in improving the timing of future releases. If *T. minutum* can be released early enough in the oviposition curve of the budworm, a large number of parasitoids will emerge and be active 14 to 19 days later at the end of the oviposition curve. We can benefit, thereby, by having 'superior' parasitoids in the field without the cost of additional releases.

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Zusammenfassung

Einflußfaktoren auf die Massenfreisetzung von Trichogramma minutum Riley gegen den Wickler Choristoneura fumiferana (Clemens)

In den Wäldern der borealen Zone Kanadas wurden 1982 und 1983 Massenfreisetzungen von *T. minutum* zur biologischen Bekämpfung von *Choristoneura fumiferana* durchgeführt. Die wichtigsten Einflußfaktoren auf die Eiparasitierungsrate waren der Zeitpunkt der Freisetzung, die Anzahl freigesetzter Parasitoide und die örtlichen Witterungsbedingungen. Weniger wichtig waren der Futtervorrat für die Parasitoiden-Weibchen, die vertikale Verteilung der Wirtseier im Bestand, Intensität der Sonneneinstrahlung und Wirtsdichte. Die Baumart, an der die Eier vom Wirt abgelegt wurden, war ohne Einfluß auf die Parasitierung. Parasitoide, die bei unterschiedlichen Temperaturen und in verschiedenen Wirtseiern gezüchtet wurden, unterschieden sich hinsichtlich biologischer Eigenschaften und beeinflußten dadurch den Erfolg der Freilandfreisetzungen. Die geographischen Herkünfte von *T. minutum* sind für die spätere Freisetzung von geringerer Bedeutung als die jeweiligen Aufzuchtsbedingungen, da innerhalb der Herkünfte große individuelle Unterschiede bestehen.

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